Journal of Epidemiology and Biostatistics (2000) Vol. 5, No. 3, 153–160

Oxychlordane and *trans*-nonachlor in breast adipose tissue and risk of female breast cancer

T ZHENG[1], TR HOLFORD[1], J TESSARI[2], ST MAYNE[1], SH ZAHM[3], PH OWENS[1], B ZHANG[4], B WARD[1], D CARTER[1], Y ZHANG[1], W ZHANG[1], R DUBROW[1] and P BOYLE[5]

[1]*Yale University School of Medicine and Yale Cancer Center, New Haven, USA*
[2]*Colorado State University, Colorado, USA*
[3]*National Cancer Institute, Maryland, USA*
[4]*McGill University, Montreal, Canada,*
[5]*European Institute of Oncology, Milan, Italy*

Background Organochlorine compounds, including organochlorine pesticides, have been suggested by some, but not all, studies to be associated with female breast-cancer risk. So far, studies relating organochlorine compounds and breast-cancer risk have mainly focused on polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) as risk factors for female breast cancer. This paper examines the hypothesis that environmental exposure to *trans*-nonachlor (TNC) and oxychlordane (OCD), a major metabolite of the insecticide chlordane, increases the risk of female breast cancer.

Methods A total of 304 histologically-confirmed, incident primary breast-cancer patients and 186 histologically-confirmed incident benign breast-disease controls were included in the study between 1994 and 1997. Breast adipose tissue not needed for diagnostic purposes was collected and analysed for TNC, OCD and other organochlorine compounds. A standardised, structured questionnaire was used to obtain information on major known, or suspected, risk factors for breast cancer.

Results The age and lipid-adjusted geometric mean adipose-tissue levels of OCD were similar between the cases [36.4 p.p.b., 95% confidence interval (CI) 34.7–38.2 p.p.b.] and controls (38.0 p.p.b., 95% CI 35.7–40.6 p.p.b.). The age and lipid-adjusted geometric mean adipose-tissue levels of TNC between the cases (55.5 p.p.b., 95% CI 52.6–58.5 p.p.b.) and controls (58.1 p.p.b., 95% CI 54.2–62.3 p.p.b.) were also similar. There was no association between breast-cancer risk and mean adipose-tissue levels of OCD and TNC. The covariate-adjusted odds ratio (OR) was 0.7 (95% CI 0.4–1.3) for OCD and 1.1 (95% CI 0.6–1.9) for TNC, when the highest quartile was compared with the lowest. The risk also did not vary based on oestrogen or pro-gesterone receptor status or menopausal status.

Discussion We found no significantly increased risk of breast cancer associated with breast adipose-tissue levels of OCD or TNC; this is consistent with recent epidemiological studies, indicating that environmental exposure to organochlorine compounds does not have an overall significant impact on breast-cancer risk.

Keywords breast cancer, chlordane, epidemiology, oxychlordane, *trans*-nonachlor.

Introduction

Organochlorine compounds, including organochlorine pesticides, have recently been suggested to be associated with female breast-cancer risk[1–3]. It is biologically plausible that exposure to these environmental contaminants may increase breast-cancer risk, because some of the organochlorine compounds are animal carcinogens, oestrogenically-active and inducers of cytochrome P450 mixed-function oxidase enzymes, which are intimately involved in steroid hormone metabolism[4–10].

So far, studies relating organochlorine compounds and breast-cancer risk have mainly focused on polychlorinated biphenyls (PCBs) and dichlorodiphenyl trichloroethane (DDT) as risk factors for female breast cancer[11–16]. Two small studies[17–18] have examined the association between oxychlordane (OCD) and *trans*-nonachlor (TNC) and breast-cancer risk. OCD is a major oxidative metabolite of the insecticide chlordane. TNC is a component of technical chlordane and technical heptachlor. Chlordane and heptachlor were used

Correspondence to: T Zheng, 129 Church Street, Suite 700-703, New Haven, CT 06510, USA.

Received 18 November 1998
Revised 3 May 1999
Accepted 5 August 1999

primarily for the protection of buildings, lawns and gardens from soil insects and termites. Heptachlor was also used to control mosquitoes[19,20]. Chlordane was first produced commercially in the USA in 1947, as an insecticide. Heptachlor was isolated from technical chlordane. Since 1988, the use of chlordane in the USA has been suspended. Use of heptachlor has also been subject to restrictions. Nevertheless, significant exposure to chlordane, heptachlor and their metabolites has occurred, due to their extensive past and present use, and their highly persistent nature in the environment. Residues of these compounds have been detected throughout the food chain and in most human tissues examined[17–26].

Dewailly *et al.*[17] found an insignificantly higher (p = 0.07) mean breast adipose-tissue level of TNC in nine oestrogen-receptor positive (ER^+) breast-cancer cases (50.3 p.p.b.) than in 17 benign breast-disease (BBD) controls (42.5 p.p.b.). OCD was also found to be insignificantly higher in the ER^+ breast-cancer cases (38.9 p.p.b.) than in BBD controls (31.1 p.p.b.). Falck *et al.*[18] reported insignificantly higher breast adipose-tissue levels of OCD and heptachlorepoxide in 20 breast-cancer cases (136 p.p.b.) than in 20 BBD controls (121 p.p.b.). However, TNC was found to be insignificantly lower among the cases (103 p.p.b.) than in the controls (118 p.p.b.).

Considering their oestrogenic activities and the widespread exposure to chlordane and heptachlor and their metabolites, and realising their potential to be risk factors for breast cancer, we examined the relationship between breast adipose-tissue levels of OCD, TNC and breast-cancer risk, in a case-control study conducted in Connecticut between January 1, 1994 and December 31, 1997. Information on major potential confounders was also collected, through face-to-face interviews by trained interviewers.

Materials and methods

As described elsewhere[27–29], the cases and controls were women aged 40–79 who had breast-related surgery at Yale-New Haven Hospital (YNHH), New Haven, Connecticut, USA. Cases were histologically-confirmed, incident primary breast-cancer patients (ICD-O, 174.0–174.9). Controls were patients with histologically-confirmed incident BBD (excluding atypical hyperplasia, including a diagnosis of normal breast tissue). Cases and controls had no previous diagnosis of cancer, with the exception of non-melanoma skin cancer. A total of 490 women (304 cases and 186 controls) were enrolled in the study.

We identified potential cases and controls from the computer files maintained by the Surgical Pathology Department at YNHH, which contain information on all breast-related surgeries at YNHH. We selected all patients who met the study eligibility requirements, as described above, and whose breast pathology specimen had a sufficient amount (0.4 g) of residual breast adipose tissue for chemical analyses. We then sought physicians' approval to proceed with patient contact and sent letters to physician-approved patients, asking for their participation in the study. Eligible patients who agreed to participate were then interviewed by a trained study interviewer, in a location convenient for the patient. Information on reproductive, lactation and past medical histories, occupation and demographic factors was also obtained through in-person interview by trained study interviewers, using a standardised, structured questionnaire. The dietary information was collected through a scannable semi-quantitative food-frequency questionnaire developed by the Fred Hutchinson Cancer Research Center. Each subject was asked to characterise her usual diet in the year before she had the biopsy or interview. The participation rates for those eligible patients were 79% for the cases and 74% for the controls.

Carcinomas were histologically confirmed by the study pathologist (DC) and staged according to the TNM system (primary tumour–regional nodes–metastases system). BBD was classified and grouped according to the 1985 Pathologists' Consensus Statement, as proliferative or nonproliferative disease. Patients for whom no abnormality was found were classified in the nonproliferative group. Earlier studies suggest that environmental oestrogens may only affect the incidence of hormone-responsive breast cancer[17,30]. We therefore also collected information on oestrogen receptor (ER) and progesterone receptor (PR) levels, which were measured immunohistochemically at the Pathology Department of YNHH[31]. Both ER and PR status were considered to be positive with an H-score > 75, as described by McCarty *et al*[31].

Breast adipose tissue not needed for diagnostic purposes was collected and placed into a glass vial on ice, coded and stored at −84 °C until it was mailed in batches to the study laboratory at Colorado State University, where it remained frozen until analysis. Tissue samples were analysed in batches of 10, with each batch having approximately six cases and four controls. Laboratory personnel were blind to the case-control status of all samples being analysed. The laboratory method for analysing OCD and TNC in breast adipose tissue has been described elsewhere[32]. Briefly, the method involved extraction in hexane, separation of the organochlorine pesticides from the PCBs and purification of the sample using Florisil chromatography. Identification and quantification of the compounds was achieved using gas chromatography. The quantitation limit of this method is 10 p.p.b. for both OCD and TNC.

All analyses were conducted under an established quality control/quality assessment program, including method spikes, reagent blanks and quality control windows. Quality control mean recovery was 83%, with a cumulative variance (CV) of 15% for OCD and 91% with a CV of 11.5% for TNC. Adipose-tissue levels of OCD and TNC were reported as parts per billion (p.p.b.), which is equivalent to pg mg^{-1} lipid. Total lipid in the sample extract was quantified gravimetrically.

Breast adipose-tissue levels of OCD and TNC were compared between all cases and all controls; among pre- and post-menopausal women; based on lactation history, cases' ER/PR status, type of breast cancer (ductal versus lobular carcinoma), BBD (proliferative versus nonproliferative disease) and stage of diagnosis (Stage 0–II versus III/IV). The distribution of OCD and TNC was skewed (as shown in Figure 1), we therefore present the median as a measure of location, and the first and third quartile cut-points (25%, 75%) as summaries of the degree of variability. The log transformation was used to better approximate the normality assumption; thus the antilog of the resulting age and lipid-adjusted means, i.e. the adjusted geometric mean, was used as a summary statistic. The statistical significance for difference among multiple means of adipose-tissue levels of OCD and TNC was calculated using a general linear model.Quartiles of adipose-tissue levels of OCD and TNC were formed based on the frequency distribution of controls. A linear logistic regression model was used to adjust for confounders when estimating the exposure/disease association. The variables included in the final model were age (< 48, 48–55, 55–65, ≥ 65 years), body mass index [(BMI) < 22, 22–24.9, 25–29.9 ≥ 30 kg m^{-2}], lifetime months of lactation (0, 1–6, > 6), age at menarche (<13, 13–15, ≥ 16 years), age at first full-term pregnancy (nulliparous, < 25, ≥ 25 years), and race (Caucasian, African–American, and others). The aforementioned confounders (other than race) were also controlled for as continuous variables, rather than as categorical variables. The conclusion, however, was essentially the same. Odds ratios (OR) and 95% confidence intervals (CI) were calculated using SAS statistical software[33].

Results

As shown in Table 1, cases (mean age 56.3 years) were older than the controls (52.6 years). Since age was also associated with body burden of organochlorine compounds, it was a potential confounder and was therefore controlled in all subsequent analyses. Cases tended to have higher dietary fat intakes, shorter duration of lifetime lactation and be of an older age at first full pregnancy.

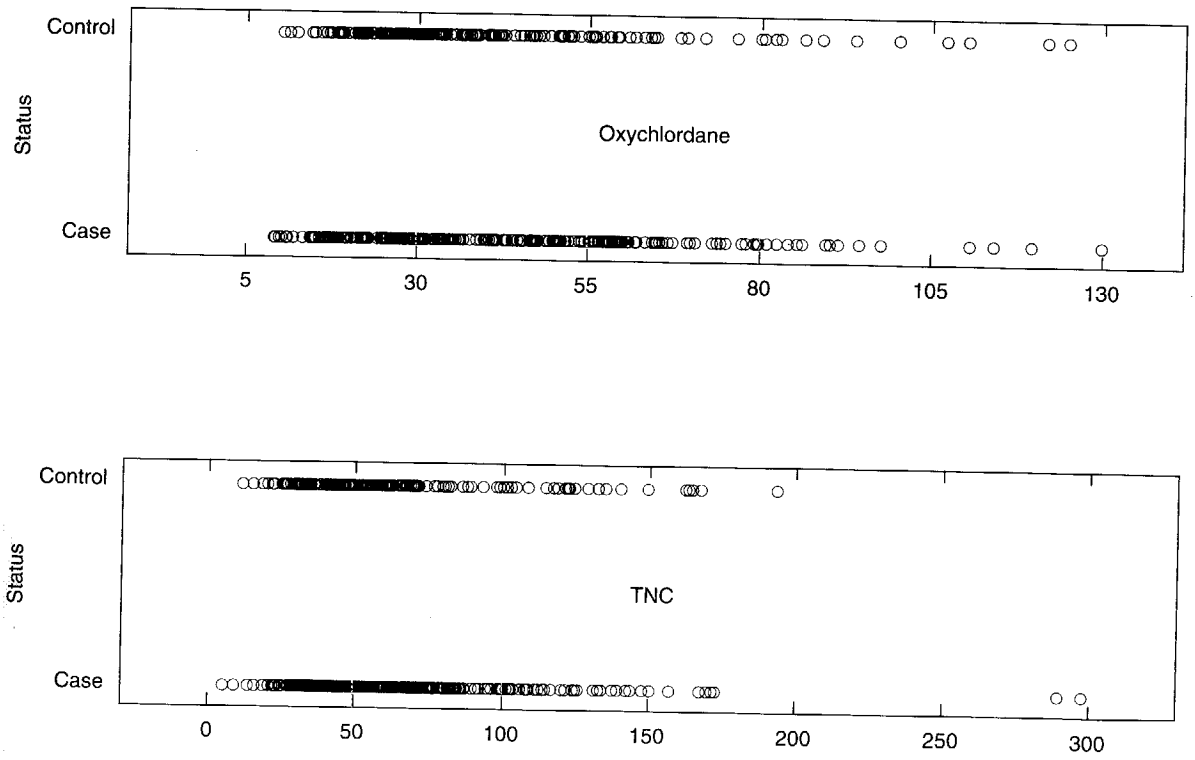

Fig. 1 The distribution of the lipid-adjusted breast adipose-tissue levels of oxychlordane and trans-nonachlor (p.p.b.) by case and control status.

As shown in Table 2, the age and lipid-adjusted geometric mean adipose-tissue levels of OCD were similar between the cases (36.4 p.p.b., 95% CI 34.7–38.2 p.p.b) and controls (38.0 p.p.b., 95% CI 35.7–40.6 p.p.b.). Adipose-tissue levels of TNC between the cases (55.5 p.p.b., 95% CI 52.6–58.5 p.p.b.) and controls (58.1 p.p.b., 95% CI 54.2–62.3 p.p.b.) were also similar. The adipose-tissue levels of both OCD and TNC did not differ between the cases and controls among either pre- or post-menopausal women (data not shown).

Cases and controls did not differ significantly in the age and lipid-adjusted geometric mean adipose-tissue levels of OCD or TNC by ER or PR status (Tables 3 and 4). The medians, and the first and the third quartile cut-points (25%, 75%) for adipose-tissue levels of OCD and TNC were also similar between the cases and controls by ER and PR status.

We further examined the geometric mean difference by ER status for those < age 50 and for those ≥ 50, since ER status is generally correlated with age at diagnosis. We found that the difference in the age and lipid-adjusted geometric means of either OCD or TNC between the controls and ER^+ or ER^- cases was not statistically significant for either age group.

Further analyses, by cancer histology (ductal and lobular carcinoma), type of BBD (proliferative and nonproliferative BBD/normal tissue) and stage at diagnosis (Stage 0–II versus III/IV), showed no significant difference in mean adipose-tissue levels of both OCD and TNC (data not shown). Results from multivariate analyses showed that there was no association between breast-cancer risk and breast adipose-tissue levels of OCD and TNC (Table 5). The covariate-adjusted OR was 0.7 (95% CI 0.4–1.3) for OCD and 1.1 (95% CI 0.6–1.9) for TNC when the highest quartile was compared with the lowest. Further stratification by lactation history also showed no increased risk of breast cancer associated with breast adipose-tissue levels of OCD and TNC (data not shown).

Table 1 Means or proportions for characteristics of cases and controls

Characteristic	Cases (n = 304)	Controls (n = 186)	P[a]
Age (years)	56.3	52.6	< 0.01
BMI (kg m^{-2})	26.2	27.1	0.41
Age at menarche (years)	12.6	12.5	0.52
Nulliparous (%)	12.2	15.1	0.3
Age at first full pregnancy[b] (years)	25.1	24.9	0.26
Lactation (months)[b]	5.3	6.5	0.05
Fat intake (g day^{-1})	63.6	62.2	0.53
Race (% Caucasian)	87.5	84.4	0.33

[a]χ^2 test for proportions and Wilcoxon two-sample test for means.
[b]Among parous women only.

Table 2 Lipid-adjusted adipose-tissue levels of oxychlordane and *trans*-nonachlor (p.p.b.) among breast-cancer cases and BBD controls

	Number of subjects	Median (25%, 75%)[a]	Age adjusted geometric mean	95% CI	P[b]
Oxychlordane					
Cases	304	36.3 (26.1, 51.7)	36.4	34.7–38.2	0.38
Controls	186	33.5 (26.0, 47.4)	38.0	35.7–40.6	
***Trans*-nonachlor**					
Cases	304	54.9 (39.0, 79.8)	55.5	52.6–58.5	0.33
Controls	186	52.9 (36.4, 71.0)	58.1	54.2–62.3	

[a]The cut-points at the first quartile (25%) and the third quartile (75%) for the lipid adjusted breast adipose-tissue levels of oxychlordane or *trans*-nonachlor.
[b]P values for test of geometric means between cases and controls adjusting for age using general linear model.

Table 3 Lipid-adjusted adipose-tissue levels of oxychlordane (p.p.b.) for breast-cancer cases and controls by ER and PR status

Receptor status	Number of subjects	Median (25%, 75%)[a]	Age adjusted geometric mean	95% CI	P[b]
Controls	186	33.5 (26.0, 47.4)	38.1	35.7–40.5	
Cases					
ER$^+$	157	37.9 (28.2, 54.0)	37.2	34.7–39.8	0.65
ER$^-$	126	34.7 (24.8, 50.5)	36.5	33.8–39.4	0.42
Unknown	21	30.3 (22.9, 42.9)	31.5	26.2–37.8	0.07
PR$^+$	116	36.7 (25.0, 51.7)	34.6	32.0–37.4	0.06
PR$^-$	128	36.2 (25.9, 52.0)	37.6	34.8–40.6	0.88
Unknown	60	36.4 (28.3, 50.5)	37.0	33.3–41.2	0.79

[a]The cut-points at the first quartile (25%) and the third quartile (75%) for the lipid-adjusted breast adipose-tissue levels of oxychlordane or *trans*-nonachlor.

[b]P values for test of geometric means between cases and controls adjusting for age using general linear model.

Table 4 Lipid-adjusted adipose-tissue levels of *trans*-nonachlor (p.p.b.) in breast-cancer cases and controls by oestrogen and progesterone receptor status

Receptor status	Number of subjects	Median (25%, 75%)[a]	Age adjusted geometric mean	95% CI	P[b]
Controls	186	52.9 (36.4, 71.0)	58.1	54.6–62.3	
Cases					
ER$^+$	157	58.5 (40.0, 85.2)	56.4	48.7–65.3	0.56
ER$^-$	126	51.2 (38.7, 71.3)	54.8	50.5–59.5	0.28
Unknown	21	49.4 (37.7, 75.0)	53.6	44.3–64.8	0.43
PR$^+$	116	50.6 (36.1, 85.2)	52.3	48.0–57.0	0.05
PR$^-$	128	56.1 (39.6, 75.4)	56.5	52.0–61.3	0.62
Unknown	60	59.0 (42.7, 77.3)	59.2	52.9–66.2	0.75

[a]The cut-points at the first quartile (25%) and the third quartile (75%) for the lipid-adjusted breast adipose-tissue levels of oxychlordane or *trans*-nonachlor.

[b]P values for test of geometric means between cases and controls adjusting for age using general linear model.

Table 5 Breast-cancer risk associated with breast adipose-tissue levels (ppb) of oxychlordane and *trans*-nonachlor

	Cases	Controls	OR_1	95% CI	OR_2	95% CI
Oxychlordane						
<26.0	75	46	1.0		1.0	
26.0–33.6	62	47	0.7	0.4–1.3	0.7	0.4–1.2
33.7–47.5	71	46	0.8	0.4–1.3	0.7	0.4–1.2
47.6–	96	47	0.8	0.5–1.4	0.7	0.4–1.3
P for trend			0.30		0.29	
***Trans*-nonachlor**						
<36.4	65	46	1.0		1.0	
36.4–53.1	79	47	1.2	0.7–2.1	1.2	0.7–2.1
53.2–71.0	57	46	0.8	0.4–1.3	0.7	0.4–1.3
71.1	103	47	1.2	0.7–2.1	1.1	0.6–1.9
P for trend			0.41		0.44	

OR_1: adjusted only for age. OR_2: adjusted for age (< 48, 48–, 55–, ≥ 65 years), BMI (< 22, 22–24.9, 25–29.9 ≥ 30 kg m^{-2}), lifetime months of lactation (0, 1–6, > 6), age at menarche (< 13, 13–15, ≥ 16 years), age at first full-term pregnancy (nulliparous, < 25, ≥ 25 years), and race (Caucasian, African–American and others).

Discussion

The results from this study do not support an overall association between breast adipose-tissue levels of OCD and TNC and the risk of female breast cancer. The association also did not differ by menopausal status, or histologic type, disease stage at diagnosis, or by history of lactation. The age and lipid-adjusted mean adipose-tissue levels of OCD and TNC were also not significantly different between the cases and controls by ER and PR status.

These results are consistent with recent studies suggesting that environmental exposure to various environmental oestrogens does not seem to have an overall significant impact on the risk of female breast cancer. In recent reports DDE, an environmental pollutant with greater oestrogenic activity than OCD or TNC, was not found to be associated with breast-cancer risk[13–16]. In fact, an inverse association between breast adipose-tissue levels of DDE and breast-cancer risk was reported[14], while several earlier smaller studies indicated an increased risk associated with environmental exposure to DDT and its most stable metabolite, DDE[11,17,18]. Studies of other organochlorine pesticides, including HCB, HCH and Mirex, have also produced inconclusive results[16–18,34,35].

One possible explanation for the lack of association between environmental oestrogens and breast-cancer risk, in this and other recent studies, is that most organochlorine pesticides and other environmental oestrogens are very weak oestrogens, usually hundreds to thousands of times less active than oestradiol[36]. There is therefore little chance that environmental oestrogens could exert an important oestrogenic effect[37]. Another potential explanation for the overall lack of association is that, other than the ubiquitous synthetic oestrogens in the human environment, there is also a sea of natural and synthetic antioestrogens, which may negate any effects from environmental oestrogens, hence the net effect may be zero.[37] It should be pointed out that these arguments are not universally accepted. For example, some argue that environmental oestrogens may be able to enter cells more freely than endogenous oestrogen[4]. This would greatly increase their availability and the biological activity of environmental oestrogens, relative to similar blood concentration of endogenous oestrogen — most of which is inhibited from entering cells by binding to oestrogen-binding plasma protein[38]. Results from other studies also suggest that exposure and the extent of exposure to various environmental oestrogens and anti-oestrogens varies by populations and by the actual exposures[39–41]. These effects may therefore not cancel out. The potentially unbalanced exposure to these oestrogens and anti-oestrogens may still increase or decrease the risk of breast cancer.

Experimental studies have provided sufficient evidence to suggest the carcinogenicity of heptachlor and chlordane, the parent compound of oxychlordane. The increase in cancer cases, however, is mainly in the incidence of hepatocellular neoplasms and thyroid follicular-cell neoplasms[42]. Lung cancer mortality was slightly elevated in two cohort studies of pesticide applicators and one of chlordane/heptachlor manufacturers. Small excess risks were also observed for other cancers, such as leukemia, non-Hodgkin's lymphoma and soft-tissue sarcoma, and cancers of the brain, skin, bladder and stomach, as reviewed by others[42]. No studies have, however, reported an increased risk of breast cancer associated with either occupational or environmental exposure to chlordane or heptachlor.

A pilot study by Dewailly *et al.*[17] reported nonsignificantly higher breast adipose-tissue levels of OCD (38.9 p.p.b.) and TNC (50.3 p.p.b.) for nine ER^+ breast cancer patients, compared with 17 controls (31.3 p.p.b. for OCD and 42.5 p.p.b. for TNC). The authors suggested that exposure to oestrogenic organochlorines may only affect the incidence of hormone-responsive breast cancer. In this study, however, we found that the age and lipid-adjusted geometric mean adipose-tissue levels of OCD or TNC were quite comparable between the cases and controls by ER or PR status. Therefore, our results do not support the hypothesis raised by Dewailly's study.

In interpreting the results from our study, several potential limitations need to be considered. One potential limitation of the study is its use of BBD patients as controls. If there were an association between OCD and TNC and BBD, it could lead to an under-estimation of the association between these organochlorine compounds and breast-cancer risk. However, use of BBD patients as controls in this study does not seem to be the explanation for the observed lack of association, since the mean adipose-tissue levels of OCD and TNC were similar for women diagnosed with proliferative and nonproliferative BBD or normal tissue (data not shown). The mean adipose-tissue levels of OCD and TNC for both types of controls were also not significantly different from the cases.

Another potential limitation of the study is that breast adipose-tissue levels of OCD and TNC may be affected by case status. In particular, the tissue levels of OCD and TNC for late-stage patients may be affected by mobilisation of energy from fat stores. However, in our study, only 20 breast cancer patients were diagnosed with Stage III/IV disease and exclusion of these patients from the analyses had no significant impact on the observed association.

Table 1 shows that there was a difference between the mean age of the cases (56.3 years) and controls (52.6 years). This age difference, however, cannot be used to

explain the observed negative association. Age was found to be positively associated with body burden of OCD (r = 0.45) and TNC (r = 0.46), and therefore controlled for throughout the data analyses. If residual confounding from an age difference between the cases and controls has any impact on the observed effect, it should cause a false positive association, rather than a negative one.

Another concern is the potential statistical power of the study. While we included a sample size of 490 to examine the relationship between OCD and TNC in breast adipose-tissue and breast-cancer risk, the study power may be still limited, especially with further stratification of the subjects by histologic type, ER and PR status, menopausal status and lactation history.

In summary, we found no overall significantly-increased risk of breast cancer associated with breast adipose-tissue levels of OCD or TNC, which is consistent with the most recent epidemiological studies indicating that environmental exposure to organochlorine compounds does not have an overall significant impact on breast-cancer risk. Our study also does not support the hypothesis that exposure to oestrogenic organochlorines affects the incidence of hormone-responsive breast cancer.

Acknowledgments

The authors would like to thank the study participants for contributing their time, and for their cooperation, as well as the physicians, nurses and related personnel at the Yale-New Haven Hospital who assisted with the study. Thanks also to Drs Letitia Dedios and Christine Howe at the Yale Cancer Center Shared Resources for their assistance in the collection of study materials. Special thanks to Drs Kumiko Iwamoto, Gwen Collman and G. Iris Obrams at the National Institutes of Health for their support and guidance during the study.

References

1 Wolff MS, Weston A. Breast-cancer risk and environmental exposures. *Environ Health Perspect* 1997;105 Suppl 4:891–96.

2 Davis DL, Bradlow HL, Wolff M, *et al.* Medical hypothesis: Xenooestrogens as preventable causes of breast cancer. *Environ Health Perspect* 1993;101:372–7.

3 Lopez-Carrillo L, Torres-Arreola L, Torres-Sanchez L, *et al.* Is DDT use a public health problem in Mexico? *Environ Health Perspect* 1996;104:584–8.

4 Colburn T, vom Saal FS, Soto AM. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environ Health Perspect* 1993;101:378–84.

5 Houghton DL, Ritter L. Organochlorine residues and risk of breast cancer. *J Am Coll Toxicol* 1995;14:71–89.

6 Sharpe RM, Skakkebaek NE. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? *Lancet* 1993;341:1392–5.

7 Wolff MS, Toniolo PG. Environmental organochlorine exposure as a potential etiologic factor in breast cancer. *Environ Health Perspect* 1995;103:141–5.

8 Klotz DM, Beckman BS, Mill SM, *et al.* Identification of environmental chemicals with oestrogenic activity using a combination of *in vitro* assays. *Environ Health Perspect* 1996;104:1084–9.

9 Safe S. Toxicology, structure–function relationship, and human and environmental health impacts of polychlorinated biphenyls: progress and problems. *Environ Health Perspect* 1992;100:259–68.

10 Robison AK, Sirbasku DA, Stancel GM. DDT supports the growth of an oestrogen-responsive tumor. *Toxicol Lett* 1985;27:109–13.

11 Wolff MS, Toniolo PG, Lee EW, *et al.* Blood levels of organochlorine residues and risk of breast cancer. *J Natl Cancer Inst* 1993;85:648–52.

12 Krieger N, Wolff MS, Hiatt RA, *et al.* Breast cancer and adipose-tissue levels of organochlorines: a prospective study among white, black, and Asian women. *J Natl Cancer Inst* 1994;86:589–99.

13 Hunter DJ, Hankinson SE, Laden F, *et al.* Plasma organochlorine levels and the risk of breast cancer. *N Engl J Med* 1997;337:1253–8.

14 Van 't Veer P, Lobbezoo IE, Martin-Moreno JM, *et al.* DDT (dicophane) and postmenopausal breast cancer in Europe: case-control study. *Br Med J* 1997;315: 81–5.

15 Lopez-Carrillo L, Blair A, Lopez-Cervantes M, *et al.* Dichlorodiphenyltrichloroethane adipose-tissue serum levels and breast-cancer risk: a case-control study from Mexico. *Cancer Res* 1997;57:3728–32.

16 Moysich KB, Ambrosone CB, Vena JE, *et al.* Environmental organochlorine exposure and postmenopausal breast-cancer risk. *Cancer Epidemiol Biomark Prev* 1998;7:181–8.

17 Dewailly E, Dodin S, Verreault R, *et al.* High organochlorine body burden in women with oestrogen receptor-positive breast cancer. *J Natl Cancer Inst 1994*; 86: 232–4.

18 Falck F, Ricci A, Wolff MS, *et al.* Pesticides and polychlorinated biphenyl residues in human breast lipids and their relation to breast cancer. *Arch Environ Health* 1992;47:143–6.

19 World Health Organisation. *Health and safety guide for heptachlor*. Geneva, Switzerland:WHO, 1988.

20 World Health Organisation. *Health and safety guide for chlordane*. Geneva, Switzerland:WHO, 1988.

21 Brock JW, Melnyk LJ, Caudill SP, *et al.* Serum levels of several organochlorine pesticides in farmers correspond with dietary exposure and local use history. *Toxicol Ind Health* 1998;14:275–89.

22 Rogan WJ, Ragan NB. Chemical contaminants, pharmacokinetics, and the lactating mother. *Environ Health Perspect* 1994;102:89–95.

23 Gammon MD, Wolff MS, Neugut AI, *et al.* Temporal variation in chlorinated hydrocarbons in healthy women. *Cancer Epidemiol Biomarkers Prev* 1997;6:327–32.

24 Mattison DR, Wohlleb J, To T, *et al.* Pesticide concentrations in Arkansas breast milk. *J Ark Med Soc* 1992;88:553–7.

25 Murphy R, Harvey C. Residues and metabolites of selected persistent halogenated hydrocarbons in blood specimens from a general population survey. *Environ Health Perspect* 1985;60:115–20.
26 Stehr-Green PA, Wohlleb JC, Royce W, *et al.* An evaluation of serum pesticide residue levels and liver function in persons exposed to dairy products contaminated with heptachlor. *J Am Med Assoc* 1988;259:374–7.
27 Zheng T, Holford T, Mayne ST, *et al.* DDE and DDT in breast adipose tissue and risk of female breast cancer. *Am J Epidemiol.* 1999;150:453–8.
28 Zheng T, Holford T, Mayne ST, *et al.* ß-benzene hexachloride in breast adipose tissue and risk of breast carcinoma. *Cancer* 1999; 85:2212–8.
29 Zheng T, Holford T, Mayne ST, *et al.* Environmental exposure to hexachlorobenzene (HCB) and risk of female breast cancer in Connecticut, USA. *Cancer Epidemiol Biomark Prev* 1999;8:407–11.
30 Shekhar PVM, Werdell J, Basrur VS. Environmental oestrogen stimulation of growth and oestrogen receptor function in preneoplastic and cancerous human breast cell lines. *J Natl Cancer Inst* 1997;89:1774–82.
31 McCarty KS, Miller LS, Cox EB, *et al.* Oestrogen receptor analyses. *Arch Pathol Lab Med* 1985;109:716–21.
32 Archibeque-Engle AL, Tessari JD, Winn DT, *et al.* Comparison of organochlorine pesticide and polychlorinated biphenyl residues in human breast adipose tissue and serum. *J Toxicol Environ Health* 1997;52:285–93.
33 SAS Institute. SAS/STAT user's guide. Version 6. Cary, NC: SAS Institute, 1990.
34 Mussalo-Rauhamaa H, Hasanen E, Pyysalo H, *et al.* Occurrence of beta-hexachlorocyclohexane in breast cancer patients. *Cancer* 1990;66:2124–8.
35 Wassermann M, Nogueira DP, Tomatis L, *et al.* Organochlorine compounds in neoplastic and adjacent apparently normal breast tissue. *Bull Environ Contam Toxicol* 1976;15:478–84.
36 Safe SH, Zacharewski T. Organochlorine exposure and risk for breast cancer. Etiology of breast and gynecological cancers. *Prog Clin Biol Res* 1997;396:133–45.
37 Stone R. Environmental oestrogens stir debate. *Science* 1994;265:308–10.
38 Sheehan DM, Young M. Diethylstilbestrol and estradiol binding to serum albumin and pregnancy plasma of rat and human. *Endocrinology* 1979;104:1442–6.
39 Luotamo M, Jarvisalo J, Aitio A. Assessment of exposure to polychlorinated biphenyls: analysis of selected isomers in blood and adipose tissue. *Environ Res* 1991;54:121–34.
40 Kuroki H, Masuda Y. Structures and concentrations of the main components of polychlorinated biphenyls retained in patients with yusho. *Chemosphere* 1977;6: 469–74.
41 Wolff M.S, Thornton J, Fischbein A, *et al.* Disposition of polychlorinated biphenyl congeners in occupational exposed persons. *Toxicol Appl Pharmacol* 1982;62:294–306.
42 IARC Chlordane and hepatochlor [review]. International Agency for Research on Cancer, Monographs on the evaluation of carcinogenic risks to humans in occupational exposures in insecticide application and some pesticides. Vol. 53. Lyon, IARC: 1991.